

11-2008


Comparison of Extraintestinal Pathogenic Escherichia coli Strains from Human and Avian Sources Reveals a Mixed Subset Representing Potential Zoonotic Pathogens

Timothy J. Johnson
Iowa State University

Yvonne Wannemuehler
Iowa State University, ywanne@iastate.edu

Sara J. Johnson
Iowa State University

Adam L. Stell
Follow this and additional works at: http://lib.dr.iastate.edu/vmpm_pubs

 Part of the [Bacterial Infections and Mycoses Commons](#), [Digestive System Diseases Commons](#), [Infectious Disease Commons](#), [Veterinary Microbiology and Immunobiology Commons](#), and the [Veterinary Preventive Medicine, Epidemiology, and Public Health Commons](#)

See next page for additional authors.
The complete bibliographic information for this item can be found at http://lib.dr.iastate.edu/vmpm_pubs/15. For information on how to cite this item, please visit <http://lib.dr.iastate.edu/howtocite.html>.

Comparison of Extraintestinal Pathogenic *Escherichia coli* Strains from Human and Avian Sources Reveals a Mixed Subset Representing Potential Zoonotic Pathogens

Abstract

Since extraintestinal pathogenic *Escherichia coli* (ExPEC) strains from human and avian hosts encounter similar challenges in establishing infection in extraintestinal locations, they may share similar contents of virulence genes and capacities to cause disease. In the present study, 1,074 ExPEC isolates were classified by phylogenetic group and possession of 67 other traits, including virulence-associated genes and plasmid replicon types. These ExPEC isolates included 452 avian pathogenic *E. coli* strains from avian colibacillosis, 91 neonatal meningitis *E. coli* (NMEC) strains causing human neonatal meningitis, and 531 uropathogenic *E. coli* strains from human urinary tract infections. Cluster analysis of the data revealed that most members of each subpathotype represent a genetically distinct group and have distinguishing characteristics. However, a genotyping cluster containing 108 ExPEC isolates was identified, heavily mixed with regard to subpathotype, in which there was substantial trait overlap. Many of the isolates within this cluster belonged to the O1, O2, or O18 serogroup. Also, 58% belonged to the ST95 multilocus sequence typing group, and over 90% of them were assigned to the B2 phylogenetic group typical of human ExPEC strains. This cluster contained strains with a high number of both chromosome- and plasmid-associated ExPEC genes. Further characterization of this ExPEC subset with zoonotic potential urges future studies exploring the potential for the transmission of certain ExPEC strains between humans and animals. Also, the widespread occurrence of plasmids among NMEC strains and members of the mixed cluster suggests that plasmid-mediated virulence in these pathotypes warrants further attention.

Disciplines

Bacterial Infections and Mycoses | Digestive System Diseases | Infectious Disease | Veterinary Microbiology and Immunobiology | Veterinary Preventive Medicine, Epidemiology, and Public Health

Comments

This article is from *Applied and Environmental Microbiology* 74, no. 22 (November 2008): 7043–7050, doi:[10.1128/AEM.01395-08](https://doi.org/10.1128/AEM.01395-08).

Authors

Timothy J. Johnson, Yvonne Wannemuehler, Sara J. Johnson, Adam L. Stell, Curt Doetkott, James R. Johnson, Kwang S. Kim, Lodewijk Spanjaard, and Lisa K. Nolan

Comparison of Extraintestinal Pathogenic *Escherichia coli* Strains from Human and Avian Sources Reveals a Mixed Subset Representing Potential Zoonotic Pathogens

Timothy J. Johnson, Yvonne Wannemuehler, Sara J. Johnson, Adam L. Stell, Curt Doetkott, James R. Johnson, Kwang S. Kim, Lodewijk Spanjaard and Lisa K. Nolan
Appl. Environ. Microbiol. 2008, 74(22):7043. DOI:
10.1128/AEM.01395-08.

Published Ahead of Print 26 September 2008.

Updated information and services can be found at:
<http://aem.asm.org/content/74/22/7043>

These include:

REFERENCES

This article cites 43 articles, 31 of which can be accessed free at: <http://aem.asm.org/content/74/22/7043#ref-list-1>

CONTENT ALERTS

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), [more»](#)

Information about commercial reprint orders: <http://journals.asm.org/site/misc/reprints.xhtml>
To subscribe to to another ASM Journal go to: <http://journals.asm.org/site/subscriptions/>

Comparison of Extraintestinal Pathogenic *Escherichia coli* Strains from Human and Avian Sources Reveals a Mixed Subset Representing Potential Zoonotic Pathogens[▽]

Timothy J. Johnson,^{1,2} Yvonne Wannemuehler,² Sara J. Johnson,² Adam L. Stell,¹ Curt Doetkott,³ James R. Johnson,⁴ Kwang S. Kim,⁵ Lodewijk Spanjaard,⁶ and Lisa K. Nolan^{2*}

Department of Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, Minnesota 55108¹; Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa 50011²; Information Technology Services, North Dakota State University, Fargo, North Dakota 58105³; Mucosal and Vaccine Research Center, VA Medical Center, and Department of Medicine, University of Minnesota, Minneapolis, Minnesota⁴; Division of Pediatric Infectious Diseases, School of Medicine, Johns Hopkins University, Baltimore, Maryland 21287⁵; and Netherlands Reference Laboratory for Bacterial Meningitis, Center of Infection and Immunity Amsterdam, Academic Medical Center, Post Box 22660, 1100 DD Amsterdam, The Netherlands⁶

Received 22 June 2008/Accepted 16 September 2008

Since extraintestinal pathogenic *Escherichia coli* (ExPEC) strains from human and avian hosts encounter similar challenges in establishing infection in extraintestinal locations, they may share similar contents of virulence genes and capacities to cause disease. In the present study, 1,074 ExPEC isolates were classified by phylogenetic group and possession of 67 other traits, including virulence-associated genes and plasmid replicon types. These ExPEC isolates included 452 avian pathogenic *E. coli* strains from avian colibacillosis, 91 neonatal meningitis *E. coli* (NMEC) strains causing human neonatal meningitis, and 531 uropathogenic *E. coli* strains from human urinary tract infections. Cluster analysis of the data revealed that most members of each subpathotype represent a genetically distinct group and have distinguishing characteristics. However, a genotyping cluster containing 108 ExPEC isolates was identified, heavily mixed with regard to subpathotype, in which there was substantial trait overlap. Many of the isolates within this cluster belonged to the O1, O2, or O18 serogroup. Also, 58% belonged to the ST95 multilocus sequence typing group, and over 90% of them were assigned to the B2 phylogenetic group typical of human ExPEC strains. This cluster contained strains with a high number of both chromosome- and plasmid-associated ExPEC genes. Further characterization of this ExPEC subset with zoonotic potential urges future studies exploring the potential for the transmission of certain ExPEC strains between humans and animals. Also, the widespread occurrence of plasmids among NMEC strains and members of the mixed cluster suggests that plasmid-mediated virulence in these pathotypes warrants further attention.

Speculation has long existed regarding a food-borne origin for extraintestinal pathogenic *Escherichia coli* (ExPEC) strains (28, 33, 42) and has spawned recent work investigating *E. coli* contaminants of food and the ExPEC strains of food-producing animals (15, 18, 24, 40). Of particular interest in this regard are avian pathogenic *E. coli* (APEC) strains that cause colibacillosis in poultry (3, 9, 35, 36, 38). Although it has been widely assumed that most APEC strains do not possess zoonotic potential, recent reports have suggested otherwise for certain groups of strains (2, 9, 29, 30, 35, 36), and some researchers have demonstrated that APEC strains and their plasmids may be transmitted to human hosts (27, 38). Recently, APEC isolates have been compared to ExPEC isolates from human urinary tract infections (UTIs) and neonatal meningitis, revealing that these “subpathotypes” have some overlap in serogroups, phylogenetic groups, virulence genotypes, and abilities to cause disease in certain animal models (9, 30, 31, 35, 36). The validity of these observations was sustained by comparison

of the first APEC genome sequence with sequenced ExPEC isolates of humans (25), which revealed that few differences existed between the sequenced APEC strain (APEC O1) and human strains. In fact, results of an *in silico* multilocus sequence typing (MLST) comparison of APEC O1 and all other sequenced *E. coli* genomes showed that APEC O1 belonged to the same sequence type (ST), ST95 (also referred to as ST29), as several well-characterized human ExPEC strains, including uropathogenic *E. coli* (UPEC) strains UTI89 and NU14 and neonatal meningitis *E. coli* (NMEC) strain RS218 (25).

While such data provide compelling evidence that APEC may be linked to human ExPEC, the results should not be overinterpreted to mean that all human ExPEC strains, or even most, are derived from APEC. APEC O1 was chosen for sequencing because it appeared to contain both UPEC- and APEC-like traits, not because it was representative of mainstream APEC (25). Regardless, other reports lend support to the idea that APEC and human ExPEC share chromosomal similarities. For instance, the *ibeA* gene, recognized for its contributions to the invasion of brain microvascular endothelial cells by human NMEC infection, was found significantly more often in APEC strains than in avian commensal strains (9, 10, 31, 34), and when *ibeA* was inactivated in the APEC

* Corresponding author. Mailing address: Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011. Phone: (515) 294-3534. Fax: (515) 294-3839. E-mail: lknolan@iastate.edu.

[▽] Published ahead of print on 26 September 2008.

TABLE 1. Results of genotyping studies^a

Gene, strain, or replicon	% of prevalence relative to the total no. of isolates (<i>n</i>)			Statistical significance of prevalence			
	UPEC (531)	NMEC (91)	APEC (452)	APEC vs human ExPEC	APEC vs UPEC	APEC vs NMEC	UPEC vs NMEC
<i>traT</i>	67.8	85.6	78.1	+	—	—	—
<i>sitA</i>	83.4	95.6	89.6	—	—	—	—
<i>iutA</i>	48.4	77.8	80.8	++	++	—	+
<i>hlyF</i>	5.6	58.9	75.4	++	++	—	++
<i>etsA</i>	6.0	61.1	67.0	++	++	—	++
<i>etsB</i>	6.0	58.9	66.8	++	++	—	++
<i>ompT</i> epi	5.6	64.4	81.6	++	++	—	++
<i>iss</i> epi	26.6	55.6	82.7	++	++	++	++
<i>iroN</i>	34.8	63.3	87.4	++	++	++	++
<i>cvaA</i>	23.4	68.9	77.4	++	++	—	++
<i>cvaB5'</i>	24.1	65.6	77.4	++	++	—	++
<i>cvaB3'</i>	22.0	61.1	68.1	++	++	—	++
<i>cvaC</i>	5.6	54.4	67.5	++	++	—	++
<i>cmi</i>	3.8	4.4	24.6	++	++	++	—
<i>cba</i>	4.0	21.1	34.3	++	++	—	++
<i>tsh</i>	2.6	31.1	52.7	++	++	+	++
<i>eitA</i>	4.3	5.6	37.2	++	++	++	—
<i>eitB</i>	4.5	5.6	37.2	++	++	++	—
UI1051	0.4	2.2	26.5	++	++	++	—
UI1024	2.4	5.6	19.7	++	++	—	—
<i>parB</i>	2.4	5.6	19.7	++	++	—	—
<i>umuC</i>	3.2	5.6	19.7	++	++	—	—
<i>adhE</i>	2.1	0.0	0.2	—	—	—	—
<i>papA</i>	54.8	28.9	7.5	++	++	++	++
<i>papC</i>	59.7	35.6	40.5	++	++	—	+
<i>papEF</i>	55.4	32.2	39.2	++	++	—	+
<i>papG1</i>	0.6	6.7	1.5	—	—	+	++
<i>papG2</i>	42.9	22.2	40.7	++	—	+	++
<i>papG3</i>	20.2	4.4	0.7	—	++	—	++
<i>kps1</i>	29.2	70.0	15.7	++	++	++	++
<i>kps2</i>	78.5	85.6	25.0	++	++	++	—
<i>kps3</i>	4.0	2.2	1.8	—	—	—	—
<i>malX</i>	68.2	56.7	15.0	++	++	++	—
<i>ireA</i>	26.0	17.8	48.0	++	++	++	—
<i>ibeA</i>	19.2	58.9	14.2	++	—	++	++
<i>gimB</i>	22.6	56.7	8.8	++	++	++	++
<i>vat</i>	62.3	74.4	33.4	++	++	++	—
<i>cnf1</i>	23.4	4.4	1.3	++	++	—	++
<i>fyuA</i>	80.6	68.9	58.2	++	++	—	—
<i>cdtB</i>	8.7	35.6	1.1	++	++	++	++
<i>bmaE</i>	1.3	2.2	0.4	—	—	—	—
<i>sfa/foc</i>	26.4	51.1	4.4	++	++	++	++
<i>hlyD</i>	34.1	3.3	0.9	++	++	—	++
<i>rfc</i>	5.3	4.4	0.4	++	—	—	—
<i>ompT</i> chrom	81.5	31.1	70.4	—	+	++	++
<i>ftiC_{H7}</i>	16.0	47.8	4.6	++	++	++	++
<i>focG</i>	14.3	2.2	0.0	++	++	++	++
<i>iha</i>	39.2	26.7	3.5	++	++	++	—
<i>afa</i>	12.6	25.6	8.2	+	—	++	+
<i>sfaS</i>	14.1	46.7	4.0	++	++	++	++
IncB/O replicon	14.5	38.9	17.9	—	—	++	++
IncFIC replicon	1.1	3.3	12.4	++	++	—	—
IncA/C replicon	0.6	0.0	3.3	++	+	—	—
IncP replicon	0.8	8.9	21.7	++	++	—	+
IncT replicon	0.0	0.0	0.9	—	—	—	—
IncK/B replicon	0.0	2.2	1.5	—	—	—	—
IncW replicon	0.2	0.0	0.0	—	—	—	—
IncFIIA replicon	3.0	1.1	24.3	++	++	++	—
IncFIA replicon	2.6	1.1	1.5	—	—	—	—
IncFIB replicon	33.5	80.0	86.9	++	++	—	++
IncY replicon	1.7	1.1	4.2	—	—	—	—
IncI1 replicon	4.5	6.7	38.3	++	++	++	—
IncX replicon	0.0	0.0	0.0	—	—	—	—
IncHI1 replicon	1.9	0.0	1.1	—	—	—	—
IncN replicon	0.2	2.2	15.0	++	++	++	—
IncHI2 replicon	0.2	0.0	4.0	++	++	—	—
IncL/M replicon	0.0	0.0	0.7	—	—	—	—
Phylo A	10.5	11.1	36.9	++	++	++	—
Phylo B1	4.5	2.2	15.9	++	++	+	—
Phylo B2	62.7	76.7	17.3	++	++	++	—
Phylo D	22.2	11.1	29.9	++	—	+	—

^a Values shown for results of genotyping are given in percentages. Two-way comparisons were performed for each gene, strain, or replicon studied between the different groups examined, using Fisher's exact test. For each comparison, a *P* value of <0.05 (+) was considered statistically significant, and a *P* value of <0.01 (++) was also considered statistically significant, while a *P* value of >0.05 (—) was not considered statistically significant. epi, episomal; chrom, chromosomal; Phylo, phylotype.

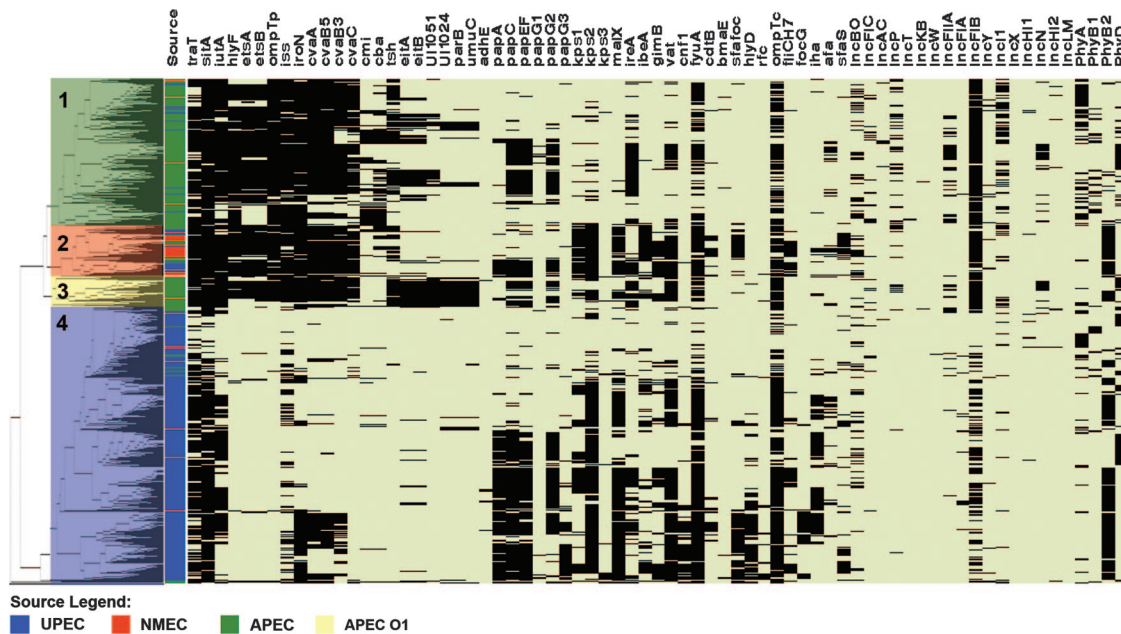


FIG. 2. Results of cluster and discriminant analyses based on the traits examined. From left to right, the dendrogram was constructed based upon the cluster analysis of common traits, and cluster numbers (1 to 4) were discerned using a cutoff based upon overall virulence genotype; the source column indicates the origin of an isolate; the following columns depict individual PCR results for the presence (black) or absence (light green) of plasmid-carried genes, chromosomal genes, plasmid replicons, and phylogenetic type. *ompTp*, episomal *ompT*; *iss*, episomal *iss*; *ompTc*, chromosomal *ompT*.

ilar in their possession of RepFIB and ColV virulence plasmids (Table 1). In particular, APEC and NMEC isolates did not differ significantly ($P > 0.05$) in their possession of most of the genes of the conserved PAI of ColV plasmids, including *sitA*, *iutA*, *hlyF*, *etsAB*, and *ompT* and genes of the ColV operon (21). With regard to plasmid replicon type, both APEC and NMEC isolates had a similarly high prevalence of the IncFIB plasmid replicon, with generally lower occurrences of other replicon types. The FIC, P, and I1 plasmid replicons occurred in a significantly higher proportion of APEC isolates than NMEC isolates. Chromosomal genes possessed by both groups ($P > 0.05$) included some genes of the *pap* operon (26) and *fyuA* of the yersiniabactin operon (41). However, these two groups did exhibit considerable differences in the prevalence of most other chromosomal genes, with NMEC isolates generally possessing them and APEC isolates generally not possessing them. These chromosomal differences were supported by the finding that APEC and NMEC isolates belonged to different phylogenetic groups, with most APEC isolates belonging to groups A (37%) and D (30%) and most NMEC isolates belonging to group B2 (77%). While the phylogenetic typing scheme originally described by Clermont et al. and used here is not the most discriminatory phylogenetic classification method, it has proven effective at rapidly distinguishing between pathogenic and nonpathogenic ExPEC organisms (6, 46). However, caution should be taken when interpreting such results, as more sensitive methods are available for classifying ExPEC isolates by phylogeny, such as MLST. Nevertheless, the rapid phylogenetic typing scheme was useful for the purposes of this study, when combined with virulence genotype.

UPEC isolates have different virulence genotypes than those of both APEC and NMEC. The 531 UPEC isolates examined

were significantly different from those of APEC and UPEC in many of the genes studied (Table 1). UPEC isolates possessed the ColV plasmid PAI genes at a significantly lower rate than those of APEC and NMEC, ranging from 5 to 27%. These rates excluded *iutA*, *sitA*, and *iroN*, because these genes can also occur on the UPEC chromosome (37, 44, 45). Chromosomal genes occurring at significantly different rates among the UPEC isolates examined included genes of the *pap* operon, *kps* type 1, *cnf1*, *focG*, *sfa/foc*, and IncFIB (compared to APEC and NMEC isolates); *fyuA*, *malX*, *ireA*, *kps* type 2, *vat*, IncFIC, IncP, IncFIIA, IncI1, and IncN (compared to APEC isolates); and *ireA*, *ibeA*, *gimB*, *cdtB*, *fliCH7*, *afa*, chromosomal *ompT*, *sfaS*, and IncB/O (compared to NMEC isolates). Most of the UPEC isolates examined belonged to the B2 and D phylogenetic groups.

APEC strains are different from human ExPEC strains, as a whole. Compared to human ExPEC (UPEC and NMEC) strains, the APEC strains examined were significantly different ($P < 0.01$) in nearly all of the traits examined, with the exception of genes occurring at a high rate among all groups, such as *sitA*, *traT*, chromosomal *ompT*, and those occurring at low rates among all groups, including *adhE*, the *papG* allele 3, the *kps* type 3 capsular synthesis gene, *bmaE*, and several plasmid replicons.

What traits characterize each of the ExPEC subpathotypes? Using two-way clustering, we attempted to characterize the ExPEC subpathotypes examined based upon their possession of genes/traits (Fig. 1). Again, the APEC and NMEC strains appeared to be characterized by the presence of the plasmid-carried PAI of ColV plasmids (21). The UPEC strains examined generally did not contain any of these genes. All three subpathotypes were characterized by the presence of

sitA and *traT*, while only APEC strains were characterized as containing *tsh*.

With regard to chromosome-associated traits, the APEC strains were distinguished from the UPEC and NMEC strains because they lacked most of these genes. The UPEC and NMEC strains were characterized by their possession of genes of the *pap* operon, the *kps* capsular synthesis genes (type 2 for all human ExPEC and type 1 for NMEC), the *malX* PAI marker, *vat*, and their assignment to the B2 phylogenetic group. The NMEC strains also were further characterized by their possession of *ibeA*, *gimB*, and *sfa/foc*. All three groups were characterized by their possession of *fyuA*.

Cluster analysis for gene correlations showed close relationships overall between genes of the *pap* operon, *ireA*, and chromosomal *ompT*; between genes of the conserved portion of the ColV PAI; and between several chromosomal PAI-associated genes, including the *kps* type 1 capsular synthesis gene, *ibeA*, *gimB*, *sfa/foc*, *cdtB*, and *afa*. Clustering of the subpathotypes UPEC, NMEC, and APEC based upon gene prevalence illustrates that APEC and NMEC strains shared the highest similarities to one another (Fig. 1).

Cluster analysis further defines ExPEC subpathotypes. An additional cluster analysis was performed, grouping isolates together based upon their overall possession or the absence of traits examined. Such an analysis is an excellent supplement to gene prevalence because it allows for a visualization of genetic associations among individual isolates. Four major clusters could be discerned from this analysis (Fig. 2). Clusters 1 and 3 in Fig. 2 contained mostly APEC isolates. Most of the isolates from cluster 1 belonged to the phylogenetic group A, and nearly all of the isolates in cluster 1 contained the genes of the conserved ColV PAI. Some of the isolates within cluster 1 also appeared to contain the *pap* operon, *ireA*, *vat*, chromosomal *ompT*, and *fyuA*. Isolates in this cluster contained the ColB/M operon, the ColV operon, or both. This characteristic could reflect different variants of colicin virulence plasmids that have arisen over time. Isolates from cluster 3 belonged to either the phylogenetic group B2 or D. Isolates in this cluster generally contained the genes of the conserved portion of the ColV PAI, as well as other ColV-associated genes, such as *tsh* and *eitAB*. Cluster 3 isolates generally lacked chromosomal traits.

Cluster 4 (Fig. 2) contained mostly UPEC and some NMEC isolates. Most of the isolates in cluster 4 belonged to the B2 and D phylogenetic groups. These isolates generally lacked genes of the ColV plasmid PAI but contained *traT*, *sitA*, and *iutA*. These isolates also contained the *kps* type 2 capsular synthesis gene, *malX*, *vat*, *fyuA*, and chromosomal *ompT*. Some of the cluster 4 isolates possessed the IncFIB plasmid replicon, but these isolates lacked other known plasmid replicon types. Some of the cluster 4 isolates contained *iroN* and portions of the ColV operon but not other ColV-associated genes. This characteristic could reflect the presence of a chromosomal PAI similar to that of PAI III₅₃₆ of UPEC strain 536 in these isolates (7).

Cluster analysis defines a mixed subset representing B2 strains that also contain a virulence plasmid. Cluster 2 (Fig. 2) contained a mixture of all three ExPEC subpathotypes examined. This cluster contained 108 isolates, including 39 APEC, 50 NMEC, and 19 UPEC isolates (Table 2 and Fig. 3). Nearly all of these isolates appeared to contain the ColV PAI, with the

TABLE 2. Prevalence of genes and/or traits in a mixed genotyping cluster^a

Gene, strain, or replicon	% of prevalence			
	UPEC	NMEC	APEC	Overall
% of total	8.6	3.6	54.9	10.1
<i>traT</i>	100.0	98.0	98.1	98.1
<i>sitA</i>	100.0	100.0	98.1	98.1
<i>iutA</i>	78.9	98.0	93.5	93.5
<i>hlyF</i>	94.7	94.0	88.9	88.9
<i>etsA</i>	100.0	94.0	90.7	90.7
<i>etsB</i>	94.7	92.0	89.8	89.8
<i>ompT</i> chrom	89.5	96.0	92.6	92.6
<i>iss</i> epi	89.5	80.0	88.9	88.9
<i>iroN</i>	100.0	86.0	90.7	90.7
<i>cvaA</i>	100.0	96.0	98.1	98.1
<i>cvaB5</i>	100.0	96.0	98.1	98.1
<i>cvaB3</i>	94.7	96.0	91.7	91.7
<i>cvaC</i>	89.5	88.0	88.0	88.0
<i>cmi</i>	15.8	2.0	12.0	12.0
<i>cba</i>	21.1	30.0	29.6	29.6
<i>tsh</i>	21.1	44.0	42.6	42.6
<i>eitA</i>	10.5	0.0	11.1	11.1
<i>eitB</i>	10.5	0.0	11.1	11.1
U11051	5.3	0.0	4.6	4.6
U11024	0.0	0.0	0.9	0.9
<i>parB</i>	0.0	0.0	0.9	0.9
<i>umuC</i>	0.0	0.0	0.9	0.9
<i>adhE</i>	0.0	0.0	0.0	0.0
<i>papA</i>	73.7	26.0	34.3	34.3
<i>papC</i>	73.7	30.0	40.7	40.7
<i>papEF</i>	84.2	26.0	39.8	39.8
<i>papG1</i>	0.0	0.0	0.0	0.0
<i>papG2</i>	73.7	18.0	37.0	37.0
<i>papG3</i>	5.3	0.0	0.9	0.9
<i>kps1</i>	94.7	90.0	88.9	88.9
<i>kps2</i>	100.0	100.0	98.1	98.1
<i>kps3</i>	0.0	2.0	0.9	0.9
<i>malX</i>	100.0	72.0	78.7	78.7
<i>ireA</i>	63.2	24.0	38.0	38.0
<i>ibeA</i>	31.6	80.0	71.3	71.3
<i>gimB</i>	73.7	78.0	67.6	67.6
<i>vat</i>	73.7	100.0	88.9	88.9
<i>cnfI</i>	0.0	0.0	1.9	1.9
<i>fyuA</i>	100.0	82.0	89.8	89.8
<i>cdtB</i>	5.3	56.0	28.7	28.7
<i>bmaE</i>	0.0	0.0	0.0	0.0
<i>sfa/foc</i>	21.1	76.0	50.0	50.0
<i>hlyD</i>	0.0	0.0	0.0	0.0
<i>rfe</i>	0.0	4.0	1.9	1.9
<i>ompT</i> chrom	100.0	40.0	72.2	72.2
<i>flhC_{H7}</i>	68.4	72.0	58.3	58.3
<i>focG</i>	0.0	0.0	0.0	0.0
<i>iha</i>	5.3	34.0	16.7	16.7
<i>afa</i>	0.0	40.0	18.5	18.5
<i>sfaS</i>	15.8	56.0	39.8	39.8
IncB/O replicon	10.5	48.0	25.9	25.9
IncFIC replicon	0.0	2.0	2.8	2.8
IncA/C replicon	0.0	0.0	0.0	0.0
IncP replicon	5.3	10.0	13.0	13.0
IncT replicon	0.0	0.0	0.0	0.0
IncK/B replicon	0.0	2.0	0.9	0.9
IncW replicon	0.0	0.0	0.0	0.0
IncFIIA replicon	0.0	0.0	2.8	2.8
IncFIA replicon	0.0	2.0	0.9	0.9
IncFIB replicon	68.4	92.0	84.3	84.3
IncY replicon	5.3	2.0	1.9	1.9
IncI1 replicon	15.8	2.0	20.4	20.4
IncX replicon	0.0	0.0	0.0	0.0
IncHI1 replicon	5.3	0.0	1.9	1.9
IncN replicon	0.0	0.0	0.9	0.9
IncHI2 replicon	0.0	0.0	0.9	0.9
IncL/M replicon	0.0	0.0	1.9	1.9
Phylo A	0.0	2.0	5.6	5.6
Phylo B1	0.0	0.0	1.9	1.9
Phylo B2	100.0	96.0	89.8	89.8
Phylo D	0.0	2.0	2.8	2.8

^a The cluster shown is that of cluster 2 from Fig. 1. epi, episomal; chrom, chromosomal; Phylo, phylotype.

prevalence of these genes within this cluster ranging from 88 to 99% (Table 2). About 25% of these isolates appeared to contain a plasmid variant involving genes of the ColB/M operons and *eitABC*, a putative ABC transporter system (22). Approximately one-third of the isolates from this cluster appeared to possess an intact *pap* operon, and most possessed the *kps*

Serogroup	Pathotype	Phylotype	ST05	stx1	stx2	stx3	stx4	stx5	stx6	stx7	stx8	stx9	stx10	stx11	stx12	stx13	stx14	stx15	stx16	stx17	stx18	stx19	stx20	stx21	stx22	stx23	stx24	stx25	stx26	stx27	stx28	stx29	stx30	stx31	stx32	stx33	stx34	stx35	stx36	stx37	stx38	stx39	stx40	stx41	stx42	stx43	stx44	stx45	stx46	stx47	stx48	stx49	stx50	stx51	stx52	stx53	stx54	stx55	stx56	stx57	stx58	stx59	stx60	stx61	stx62	stx63	stx64	stx65	stx66	stx67	stx68	stx69	stx70	stx71	stx72	stx73	stx74	stx75	stx76	stx77	stx78	stx79	stx80	stx81	stx82	stx83	stx84	stx85	stx86	stx87	stx88	stx89	stx90	stx91	stx92	stx93	stx94	stx95	stx96	stx97	stx98	stx99	stx100	stx101	stx102	stx103	stx104	stx105	stx106	stx107	stx108	stx109	stx110	stx111	stx112	stx113	stx114	stx115	stx116	stx117	stx118	stx119	stx120	stx121	stx122	stx123	stx124	stx125	stx126	stx127	stx128	stx129	stx130	stx131	stx132	stx133	stx134	stx135	stx136	stx137	stx138	stx139	stx140	stx141	stx142	stx143	stx144	stx145	stx146	stx147	stx148	stx149	stx150	stx151	stx152	stx153	stx154	stx155	stx156	stx157	stx158	stx159	stx160	stx161	stx162	stx163	stx164	stx165	stx166	stx167	stx168	stx169	stx170	stx171	stx172	stx173	stx174	stx175	stx176	stx177	stx178	stx179	stx180	stx181	stx182	stx183	stx184	stx185	stx186	stx187	stx188	stx189	stx190	stx191	stx192	stx193	stx194	stx195	stx196	stx197	stx198	stx199	stx200	stx201	stx202	stx203	stx204	stx205	stx206	stx207	stx208	stx209	stx210	stx211	stx212	stx213	stx214	stx215	stx216	stx217	stx218	stx219	stx220	stx221	stx222	stx223	stx224	stx225	stx226	stx227	stx228	stx229	stx230	stx231	stx232	stx233	stx234	stx235	stx236	stx237	stx238	stx239	stx240	stx241	stx242	stx243	stx244	stx245	stx246	stx247	stx248	stx249	stx250	stx251	stx252	stx253	stx254	stx255	stx256	stx257	stx258	stx259	stx260	stx261	stx262	stx263	stx264	stx265	stx266	stx267	stx268	stx269	stx270	stx271	stx272	stx273	stx274	stx275	stx276	stx277	stx278	stx279	stx280	stx281	stx282	stx283	stx284	stx285	stx286	stx287	stx288	stx289	stx290	stx291	stx292	stx293	stx294	stx295	stx296	stx297	stx298	stx299	stx300	stx301	stx302	stx303	stx304	stx305	stx306	stx307	stx308	stx309	stx310	stx311	stx312	stx313	stx314	stx315	stx316	stx317	stx318	stx319	stx320	stx321	stx322	stx323	stx324	stx325	stx326	stx327	stx328	stx329	stx330	stx331	stx332	stx333	stx334	stx335	stx336	stx337	stx338	stx339	stx340	stx341	stx342	stx343	stx344	stx345	stx346	stx347	stx348	stx349	stx350	stx351	stx352	stx353	stx354	stx355	stx356	stx357	stx358	stx359	stx360	stx361	stx362	stx363	stx364	stx365	stx366	stx367	stx368	stx369	stx370	stx371	stx372	stx373	stx374	stx375	stx376	stx377	stx378	stx379	stx380	stx381	stx382	stx383	stx384	stx385	stx386	stx387	stx388	stx389	stx390	stx391	stx392	stx393	stx394	stx395	stx396	stx397	stx398	stx399	stx400	stx401	stx402	stx403	stx404	stx405	stx406	stx407	stx408	stx409	stx410	stx411	stx412	stx413	stx414	stx415	stx416	stx417	stx418	stx419	stx420	stx421	stx422	stx423	stx424	stx425	stx426	stx427	stx428	stx429	stx430	stx431	stx432	stx433	stx434	stx435	stx436	stx437	stx438	stx439	stx440	stx441	stx442	stx443	stx444	stx445	stx446	stx447	stx448	stx449	stx450	stx451	stx452	stx453	stx454	stx455	stx456	stx457	stx458	stx459	stx460	stx461	stx462	stx463	stx464	stx465	stx466	stx467	stx468	stx469	stx470	stx471	stx472	stx473	stx474	stx475	stx476	stx477	stx478	stx479	stx480	stx481	stx482	stx483	stx484	stx485	stx486	stx487	stx488	stx489	stx490	stx491	stx492	stx493	stx494	stx495	stx496	stx497	stx498	stx499	stx500	stx501	stx502	stx503	stx504	stx505	stx506	stx507	stx508	stx509	stx510	stx511	stx512	stx513	stx514	stx515	stx516	stx517	stx518	stx519	stx520	stx521	stx522	stx523	stx524	stx525	stx526	stx527	stx528	stx529	stx530	stx531	stx532	stx533	stx534	stx535	stx536	stx537	stx538	stx539	stx540	stx541	stx542	stx543	stx544	stx545	stx546	stx547	stx548	stx549	stx550	stx551	stx552	stx553	stx554	stx555	stx556	stx557	stx558	stx559	stx560	stx561	stx562	stx563	stx564	stx565	stx566	stx567	stx568	stx569	stx570	stx571	stx572	stx573	stx574	stx575	stx576	stx577	stx578	stx579	stx580	stx581	stx582	stx583	stx584	stx585	stx586	stx587	stx588	stx589	stx590	stx591	stx592	stx593	stx594	stx595	stx596	stx597	stx598	stx599	stx600	stx601	stx602	stx603	stx604	stx605	stx606	stx607	stx608	stx609	stx610	stx611	stx612	stx613	stx614	stx615	stx616	stx617	stx618	stx619	stx620	stx621	stx622	stx623	stx624	stx625	stx626	stx627	stx628	stx629	stx630	stx631	stx632	stx633	stx634	stx635	stx636	stx637	stx638	stx639	stx640	stx641	stx642	stx643	stx644	stx645	stx646	stx647	stx648	stx649	stx650	stx651	stx652	stx653	stx654	stx655	stx656	stx657	stx658	stx659	stx660	stx661	stx662	stx663	stx664	stx665	stx666	stx667	stx668	stx669	stx670	stx671	stx672	stx673	stx674	stx675	stx676	stx677	stx678	stx679	stx680	stx681	stx682	stx683	stx684	stx685	stx686	stx687	stx688	stx689	stx690	stx691	stx692	stx693	stx694	stx695	stx696	stx697	stx698	stx699	stx700	stx701	stx702	stx703	stx704	stx705	stx706	stx707	stx708	stx709	stx710	stx711	stx712	stx713	stx714	stx715	stx716	stx717	stx718	stx719	stx720	stx721	stx722	stx723	stx724	stx725	stx726	stx727	stx728	stx729	stx730	stx731	stx732	stx733	stx734	stx735	stx736	stx737	stx738	stx739	stx740	stx741	stx742	stx743	stx744	stx745	stx746	stx747	stx748	stx749	stx750	stx751	stx752	stx753	stx754	stx755	stx756	stx757	stx758	stx759	stx760	stx761	stx762	stx763	stx764	stx765	stx766	stx767	stx768	stx769	stx770	stx771	stx772	stx773	stx774	stx775	stx776	stx777	stx778	stx779	stx780	stx781	stx782	stx783	stx784	stx785	stx786	stx787	stx788	stx789	stx790	stx791	stx792	stx793	stx794	stx795	stx796	stx797	stx798	stx799	stx800	stx801	stx802	stx803	stx804	stx805	stx806	stx807	stx808	stx809	stx810	stx811	stx812	stx813	stx814	stx815	stx816	stx817	stx818	stx819	stx820	stx821	stx822	stx823	stx824	stx825	stx826	stx827	stx828	stx829	stx830	stx831	stx832	stx833	stx834	stx835	stx836	stx837	stx838	stx839	stx840	stx841	stx842	stx843	stx844	stx845	stx846	stx847	stx848	stx849	stx850	stx851	stx852	stx853	stx854	stx855	stx856	stx857	stx858	stx859	stx860	stx861	stx862	stx863	stx864	stx865	stx866	stx867	stx868	stx869	stx870	stx871	stx872	stx873	stx874	stx875	stx876	stx877	stx878	stx879	stx880	stx881	stx882	stx883	stx884	stx885	stx886	stx887	stx888	stx889	stx890	stx891	stx892	stx893	stx894	stx895	stx896	stx897	stx898	stx899	stx900	stx901	stx902	stx903	stx904	stx905	stx906	stx907	stx908	stx909	stx910	stx911	stx912	stx913	stx914	stx915	stx916	stx917	stx918	stx919	stx920	stx921	stx922	stx923	stx924	stx925	stx926	stx927	stx928	stx929	stx930	stx931	stx932	stx933	stx934	stx935	stx936	stx937	stx938	stx939	stx940	stx941	stx942	stx943	stx944	stx945	stx946	stx947	stx948	stx949	stx950	stx951	stx952	stx953	stx954	stx955	stx956	stx957	stx958	stx959	stx960	stx961	stx962	stx963	stx964	stx965	stx966	stx967	stx968	stx969	stx970	stx971	stx972	stx973	stx974	stx975	stx976	stx977	stx978	stx979	stx980	stx981	stx982	stx983	stx984	stx985	stx986	stx987	stx988	stx989	stx990	stx991	stx992	stx993	stx994	stx995	stx996	stx997	stx998	stx999	stx1000	stx1001	stx1002	stx1003	stx1004	stx1005	stx1006	stx1007	stx1008	stx1009	stx1010	stx1011	stx1012	stx1013	stx1014	stx1015	stx1016	stx1017	stx1018	stx1019	stx1020	stx1021	stx1022	stx1023	stx1024	stx1025	stx1026	stx1027	stx1028	stx1029	stx1030	stx1031	stx1032	stx1033	stx1034	stx1035	stx1036	stx1037	stx1038	stx1039	stx1040	stx1041	stx1042	stx1043	stx1044	stx1045	stx1046	stx1047	stx1048	stx1049	stx1050	stx1051	stx1052	stx1053	stx1054	stx1055	stx1056	stx1057	stx1058	stx1059	stx1060	stx1061	stx1062	stx1063	stx1064	stx1065	stx1066	stx1067	stx1068	stx1069	stx1070	stx1071	stx1072	stx1073	stx1074	stx1075	stx1076	stx1077	stx1078	stx1079	stx1080	stx1081	stx1082	stx1083	stx1084	stx1085	stx1086	stx1087	stx1088	stx1089	stx1090	stx1091	stx1092	stx1093	stx1094	stx1095	stx1096	stx1097	stx1098	stx1099	stx1100	stx1101	stx1102	stx1103	stx1104	stx1105	stx1106	stx1107	stx1108	stx1109	stx1110	stx1111	stx1112	stx1113	stx1114	stx1115	stx1116	stx1117	stx1118	stx1119	stx1120	stx1121	stx1122	stx1123	stx1124	stx1125	stx1126	stx1127	stx1128	stx1129	stx1130	stx1131	stx1132	stx1133	stx1134	stx1135	stx1136	stx1137	stx1138	stx1139	stx1140	stx1141	stx1142	stx1143	stx1144	stx1145	stx1146	stx1147	stx1148	stx1149	stx1150	stx1151	stx1152	stx1153	stx1154	stx1155	stx1156	stx1157	stx1158	stx1159	stx1160	stx1161	stx1162	stx1163	stx1164	stx1165	stx1166	stx1167	stx1168	stx1169	stx1170	stx1171	stx1172	stx1173	stx1174	stx1175	stx1176	stx1177	stx1178	stx1179	stx1180	stx1181	stx1182	stx1183	stx1184	stx1185	stx1186	stx1187	stx1188	stx1189	stx1190	stx1191	stx1192	stx1193	stx1194	stx1195	stx1196	stx1197	stx1198	stx1199	stx1200	stx1201	stx1202	stx1203	stx1204	stx1205	stx1206	stx1207	stx1208	stx1209	stx1210	stx1211	stx1212	stx1213	stx1214	st
-----------	-----------	-----------	------	------	------	------	------	------	------	------	------	------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	----

capsule biosynthesis type 1 or 2. Many of these isolates also contained a wide variety of chromosome-carried ExPEC traits, including *malX*, *ireA*, *ibeA*, *gimB*, *vat*, *fyuA*, *sfa/foc*, *ompT*, *fliC_{H7}*, and *sfaS*. Most of these isolates possessed the IncFIB plasmid replicon.

The 108 isolates within this mixed cluster were almost exclusively members of the B2 phylogenetic group (89.8%). Within this genotyping cluster is APEC O1, a strain which has been previously sequenced and analyzed in multiple models of ExPEC infection (25). Like other isolates in this cluster, APEC O1 possesses a ColV-type virulence plasmid with its highly conserved PAI (21). This strain has been shown to cause disease in the 1-day-old chick model of avian colibacillosis and the mouse model of human UTI (T. Johnson, unpublished data) (25). APEC O1 belongs to ST95, a potentially zoonotic sequence type strain, as determined through MLST analysis of housekeeping genes (30, 31). In fact, several recently sequenced or archetypal strains belong to this ST, including UPEC strains UT189 (5) and NU14, and NMEC strain RS218 (47). These strains all contain a variety of chromosome-carried virulence factors such as those mentioned above. It was recently determined that the *svg* gene appears to be a distinguishing trait of *E. coli* strains belonging to ST95 and the B2₁ ribotype (4). When the 108 isolates from the mixed genotyping cluster in this study were analyzed for the presence of *svg*, it was found that 58% of the isolates contained this gene, suggesting their membership within the ST95 group (Fig. 3). Many of the *svg*⁺ isolates belonged to the O1, O2, or O18 serogroup, all of which have been implicated with multiple forms of ExPEC disease. This is in agreement with the work of Achtman and Plushcke (1), who identified the K1 capsule-bearing O1:K1:H7, O2:K1:H7, and O18:K1:H7 strains shown to be closely related by multilocus enzyme electrophoresis. However, the implications and occurrence of ColV plasmids among the ST95/B2₁ subgroups have not been previously explored. The results of this study suggest that the acquisition of ColV virulence plasmids by hosts with B2 phylogeny has resulted in strains such as those within the mixed genotyping cluster, with an enhanced ability to cause disease and survive in multiple environments and in the face of multiple pressures. Future work should take unbiased approaches toward determining the prevalence of ColV virulence plasmids among ST95/B2₁-positive populations.

Conclusions. This study builds upon previous work involving extensive virulence genotyping of ExPEC populations and provides some insights into the evolution of ExPEC virulence. It is apparent from this study that most APEC, UPEC, and NMEC strains are genetically distinct from one another, and thus, their classification into subpathotypes appears to be justified. Expectedly, APEC strains are characterized by the presence of ColV-like virulence plasmids in strains belonging to the A and D phylogenetic groups. UPEC and NMEC strains are characterized by their possession of chromosome-carried virulence genes, presumably on PAIs, and they belong mostly to the B2 phylogenetic group. Many NMEC strains appear to contain ColV plasmids in addition to this chromosomal background, and cluster analyses suggest that APEC and NMEC strains share many genetic similarities, and, irrespective of host source, nearly 10% of the isolates in this study belong to a genotype cluster representing the most likely zoonotic patho-

gens. Nearly 50% of the NMEC strains examined belonged to this group, but it also included APEC and UPEC strains. It is evident from this study that the distribution of ColV plasmids is not limited to any particular phylogenetic type, as they are evenly distributed among all four phylotypes. Perhaps, the acquisition of ColV virulence plasmids by B2 strains has provided them with an enhanced ability to cause disease and survive under adverse conditions. If so, such strains thus present a threat to both human and animal health, and further work is required to determine the true zoonotic potential of these strains.

ACKNOWLEDGMENTS

Funding for this study was provided by the University of Minnesota College of Veterinary Medicine (T.J.J.), Iowa State University College of Veterinary Medicine (L.K.N.), and the Office of Research and Development, Medical Research Service, Department of Veterans Affairs (J.R.J.).

REFERENCES

- Achtman, M., and G. Plushcke. 1986. Clonal analysis of descent and virulence among selected *Escherichia coli*. *Annu. Rev. Microbiol.* **40**:185–210.
- Adiri, R. S., U. Gophna, and E. Z. Ron. 2003. Multilocus sequence typing (MLST) of *Escherichia coli* O78 strains. *FEMS Microbiol. Lett.* **222**:199–203.
- Barnes, H. J., J. P. Vaillancourt, and W. B. Gross. 2003. Colibacillosis, p. 631–652. In Y. M. Saif (ed.), *Diseases of poultry*. Blackwell Publishing, Ames, IA.
- Bidet, P., A. Metais, F. Mahjoub-Messai, L. Durand, M. Dehem, Y. Aujard, E. Bingen, X. Nassif, and S. Bonacorsi. 2007. Detection and identification by PCR of a highly virulent phylogenetic subgroup among extraintestinal pathogenic *Escherichia coli* B2 strains. *Appl. Environ. Microbiol.* **73**:2373–2377.
- Chen, S. L., C. S. Hung, J. Xu, C. S. Reigstad, V. Magrini, A. Sabo, D. Blasiar, T. Bieri, R. R. Meyer, P. Ozersky, J. R. Armstrong, R. S. Fulton, J. P. Latreille, J. Spieth, T. M. Hooton, E. R. Mardis, S. J. Hultgren, and J. I. Gordon. 2006. Identification of genes subject to positive selection in uropathogenic strains of *Escherichia coli*: a comparative genomics approach. *Proc. Natl. Acad. Sci. USA* **103**:5977–5982.
- Clermont, O., S. Bonacorsi, and E. Bingen. 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl. Environ. Microbiol.* **66**:4555–4558.
- Dobrindt, U., G. Blum-Oehler, G. Nagy, G. Schneider, A. Johann, G. Gottschalk, and J. Hacker. 2002. Genetic structure and distribution of four pathogenicity islands (PAI 1536 to PAI IV536) of uropathogenic *Escherichia coli* strain 536. *Infect. Immun.* **70**:6365–6372.
- Eisen, M. B., P. T. Spellman, P. O. Brown, and D. Botstein. 1998. Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci. USA* **95**:14863–14868.
- Ewers, C., G. Li, H. Wilking, S. Kiessling, K. Alt, E. M. Antao, C. Laturnus, I. Diehl, S. Glodde, T. Homeier, U. Bohnke, H. Steinruck, H. C. Philipp, and L. H. Wieler. 2007. Avian pathogenic, uropathogenic, and newborn meningitis-causing *Escherichia coli*: how closely related are they? *Int. J. Med. Microbiol.* **297**:163–176.
- Germon, P., Y. H. Chen, L. He, J. E. Blanco, A. Bree, C. Schouler, S. H. Huang, and M. Moulin-Schouler. 2005. *ibeA*, a virulence factor of avian pathogenic *Escherichia coli*. *Microbiology* **151**:1179–1186.
- Gordon, D. M., S. E. Stern, and P. J. Collignon. 2005. Influence of the age and sex of human hosts on the distribution of *Escherichia coli* ECOR groups and virulence traits. *Microbiology* **151**:15–23.
- Johnson, J. R., and A. L. Stell. 2000. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. *J. Infect. Dis.* **181**:261–272.
- Johnson, J. R., P. L. Roberts, and W. E. Stamm. 1987. P fimbriae and other virulence factors in *Escherichia coli* urosepsis: association with patients' characteristics. *J. Infect. Dis.* **156**:225–229.
- Johnson, J. R., K. Owens, A. Gajewski, and M. A. Kuskowski. 2005. Bacterial characteristics in relation to clinical source of *Escherichia coli* isolates from women with acute cystitis or pyelonephritis and uninfected women. *J. Clin. Microbiol.* **43**:6064–6072.
- Johnson, J. R., M. A. Kuskowski, K. Smith, T. T. O'Bryan, and S. Tatini. 2005. Antimicrobial-resistant and extraintestinal pathogenic *Escherichia coli* in retail foods. *J. Infect. Dis.* **191**:1040–1049.
- Johnson, J. R., E. Oswald, T. T. O'Bryan, M. A. Kuskowski, and L. Spanjaard. 2002. Phylogenetic distribution of virulence-associated genes among *Escherichia coli* isolates associated with neonatal bacterial meningitis in the Netherlands. *J. Infect. Dis.* **185**:774–784.
- Johnson, J. R., I. Orskov, F. Orskov, P. Goulet, B. Picard, S. L. Moseley,

- P. L. Roberts, and W. E. Stamm. 1994. O, K, and H antigens predict virulence factors, carboxylesterase B pattern, antimicrobial resistance, and host compromise among *Escherichia coli* strains causing urosepsis. *J. Infect. Dis.* **169**:119–126.
18. Johnson, J. R., M. R. Sannes, C. Croy, B. Johnston, C. Clabots, M. A. Kuskowski, J. Bender, K. E. Smith, P. L. Winokur, and E. A. Belongia. 2007. Antimicrobial drug-resistant *Escherichia coli* from humans and poultry products, Minnesota and Wisconsin, 2002–2004. *Emerg. Infect. Dis.* **13**:838–846.
19. Johnson, J. R., A. L. Stell, F. Scheutz, T. T. O'Bryan, T. A. Russo, U. B. Carlino, C. Fasching, J. Kavle, L. Van Dijk, and W. Gaastra. 2000. Analysis of the F antigen-specific *papA* alleles of extraintestinal pathogenic *Escherichia coli* using a novel multiplex PCR-based assay. *Infect. Immun.* **68**:1587–1599.
20. Johnson, T. J., Y. M. Wannemuehler, and L. K. Nolan. 2008. Evolution of the *iss* gene in *Escherichia coli*. *Appl. Environ. Microbiol.* **74**:2360–2369.
21. Johnson, T. J., S. J. Johnson, and L. K. Nolan. 2006. Complete DNA sequence of a ColBM plasmid from avian pathogenic *Escherichia coli* suggests that it evolved from closely related ColV virulence plasmids. *J. Bacteriol.* **188**:5975–5983.
22. Johnson, T. J., K. E. Siek, S. J. Johnson, and L. K. Nolan. 2006. DNA sequence of a ColV plasmid and prevalence of selected plasmid-encoded virulence genes among avian *Escherichia coli* strains. *J. Bacteriol.* **188**:745–758.
23. Johnson, T. J., Y. M. Wannemuehler, J. A. Scaccianoce, S. J. Johnson, and L. K. Nolan. 2006. Complete DNA sequence, comparative genomics, and prevalence of an IncHI2 plasmid occurring among extraintestinal pathogenic *Escherichia coli* isolates. *Antimicrob. Agents Chemother.* **50**:3929–3933.
24. Johnson, T. J., Y. M. Wannemuehler, S. J. Johnson, C. M. Logue, D. G. White, C. Doetkott, and L. K. Nolan. 2007. Plasmid replicon typing of commensal and pathogenic *Escherichia coli* isolates. *Appl. Environ. Microbiol.* **73**:1976–1983.
25. Johnson, T. J., S. Kariyawasam, Y. Wannemuehler, P. Mangiamale, S. J. Johnson, C. Doetkott, J. A. Skyberg, A. M. Lynne, J. R. Johnson, and L. K. Nolan. 2007. The genome sequence of avian pathogenic *Escherichia coli* strain O1:K1:H7 shares strong similarities with human extraintestinal pathogenic *E. coli* genomes. *J. Bacteriol.* **189**:3228–3236.
26. Kariyawasam, S., T. J. Johnson, and L. K. Nolan. 2006. The *pap* operon of avian pathogenic *Escherichia coli* strain O1:K1 is located on a novel pathogenicity island. *Infect. Immun.* **74**:744–749.
27. Levy, S. B., G. B. FitzGerald, and A. B. Maccone. 1976. Spread of antibiotic-resistant plasmids from chicken to chicken and from chicken to man. *Nature* **260**:40–42.
28. Linton, A. H., K. Howe, P. M. Bennett, M. H. Richmond, and E. J. Whiteside. 1977. The colonization of the human gut by antibiotic resistant *Escherichia coli* from chickens. *J. Appl. Bacteriol.* **43**:465–469.
29. Mokady, D., U. Gophna, and E. Z. Ron. 2005. Extensive gene diversity in septicemic *Escherichia coli* strains. *J. Clin. Microbiol.* **43**:66–73.
30. Moulin-Schouleur, M., M. Reperant, S. Laurent, A. Bree, S. Mignon-Grasteau, P. Germon, D. Rasschaert, and C. Schouler. 2007. Extraintestinal pathogenic *Escherichia coli* strains of avian and human origin: link between phylogenetic relationships and common virulence patterns. *J. Clin. Microbiol.* **45**:3366–3376.
31. Moulin-Schouleur, M., C. Schouler, P. Tailliez, M. R. Kao, A. Bree, P. Germon, E. Oswald, J. Mainil, M. Blanco, and J. Blanco. 2006. Common virulence factors and genetic relationships between O18:K1:H7 *Escherichia coli* isolates of human and avian origin. *J. Clin. Microbiol.* **44**:3484–3492.
32. Pfaff-McDonough, S. J., S. M. Horne, C. W. Giddings, J. O. Ebert, C. Doetkott, M. H. Smith, and L. K. Nolan. 2000. Complement resistance-related traits among *Escherichia coli* isolates from apparently healthy birds and birds with colibacillosis. *Avian Dis.* **44**:23–33.
33. Ramchandani, M., A. R. Manges, C. DebRoy, S. P. Smith, J. R. Johnson, and L. W. Riley. 2005. Possible animal origin of human-associated, multidrug-resistant, uropathogenic *Escherichia coli*. *Clin. Infect. Dis.* **40**:251–257.
34. Rodriguez-Siek, K. E., C. W. Giddings, C. Doetkott, T. J. Johnson, and L. K. Nolan. 2005. Characterizing the APEC pathotype. *Vet. Res.* **36**:241–256.
35. Rodriguez-Siek, K. E., C. W. Giddings, C. Doetkott, T. J. Johnson, M. K. Fakhr, and L. K. Nolan. 2005. Comparison of *Escherichia coli* isolates implicated in human urinary tract infection and avian colibacillosis. *Microbiology* **151**:2097–2110.
36. Ron, E. Z. 2006. Host specificity of septicemic *Escherichia coli*: human and avian pathogens. *Curr. Opin. Microbiol.* **9**:28–32.
37. Sabri, M., M. Caza, J. Proulx, M. H. Lymberopoulos, A. Bree, M. Moulin-Schouleur, R. Curtiss III, and C. M. Dozois. 2008. Contribution of the SitABCD, MntH, and FeoB metal transporters to the virulence of avian pathogenic *Escherichia coli* O78 strain χ 7122. *Infect. Immun.* **76**:601–611.
38. Saif, Y. M. 2008. Diseases of poultry. Blackwell Publishing, Ames, IA.
39. Sannes, M. R., M. A. Kuskowski, and J. R. Johnson. 2004. Antimicrobial resistance of *Escherichia coli* strains isolated from urine of women with cystitis or pyelonephritis and feces of dogs and healthy humans. *J. Am. Vet. Med. Assoc.* **225**:368–373.
40. Schroeder, C. M., D. G. White, B. Ge, Y. Zhang, P. F. McDermott, S. Ayers, S. Zhao, and J. Meng. 2003. Isolation of antimicrobial-resistant *Escherichia coli* from retail meats purchased in Greater Washington, DC, USA. *Int. J. Food Microbiol.* **85**:197–202.
41. Schubert, S., B. Picard, S. Gouriou, J. Heesemann, and E. Denamur. 2002. *Yersinia* high-pathogenicity island contributes to virulence in *Escherichia coli* causing extraintestinal infections. *Infect. Immun.* **70**:5335–5337.
42. Shooter, R. A., E. M. Cooke, S. A. Rousseau, and A. L. Breden. 1970. Animal sources of common serotypes of *Escherichia coli* in the food of hospital patients. Possible significance in urinary-tract infections. *Lancet* **ii**:226–228.
43. Snedecor, G. W., and W. G. Cochran. 1989. Statistical methods. Iowa State University Press, Ames.
44. Sorsa, L. J., S. Dufke, J. Heesemann, and S. Schubert. 2003. Characterization of an *iroBCDEN* gene cluster on a transmissible plasmid of uropathogenic *Escherichia coli*: evidence for horizontal transfer of a chromosomal virulence factor. *Infect. Immun.* **71**:3285–3293.
45. Valvano, M. A., R. P. Silver, and J. H. Crosa. 1986. Occurrence of chromosome- or plasmid-mediated aerobactin iron transport systems and hemolysin production among clonal groups of human invasive strains of *Escherichia coli* K1. *Infect. Immun.* **52**:192–199.
46. Wirth, T., D. Falush, R. Lan, F. Colles, P. Mensa, L. H. Weiler, H. Karch, P. R. Reeves, M. C. Maiden, H. Ochman, and M. Achtman. 2006. Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol. Microbiol.* **60**:1136–1151.
47. Xie, Y., V. Kolisnychenko, M. Paul-Satyaseela, S. Elliott, G. Parthasarathy, Y. Yao, G. Plunkett 3rd, F. R. Blattner, and K. S. Kim. 2006. Identification and characterization of *Escherichia coli* RS218-derived islands in the pathogenesis of *E. coli* meningitis. *J. Infect. Dis.* **194**:358–364.